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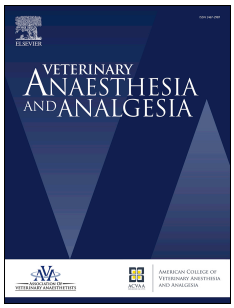

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Alfaxalone for total intravenous anaesthesia in horses.

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Running Head: Alfaxalone TIVA in horses.

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Authors' contributions

WG: study design, acquisition and interpretation of data and preparation of manuscript; KP: study design, pharmacokinetic analysis and interpretation of data and preparation of manuscript; HK: study design, acquisition and interpretation of data and preparation of manuscript; MG: plasma alfaxalone analysis and preparation of manuscript; SW: statistical

analysis and interpretation and manuscript preparation. NP: statistical analysis and interpretation and manuscript preparation.

Conflict of interest statement

Authors KP and MG work for Jurox Pty Ltd.

Abstract

Objective

To determine the suitability of alfaxalone total intravenous anaesthesia in horses and concurrently evaluate infusion rates, cardiovascular effects, pharmacokinetics and the quality of the anaesthetic recovery period.

Study Design

Prospective, experimental study.

Animals

Eight Standardbred horses.

Methods

Horses were premedicated with intravenous (IV) acepromazine (0.03 mg kg^{-1}) and xylazine (1 mg kg^{-1}) and anaesthesia was induced with guaifenesin (35 mg kg^{-1}) and alfaxalone (1 mg kg^{-1}). Anaesthesia was maintained for 180 minutes using an IV infusion of alfaxalone at a rate determined by a horse's response to a supramaximal electrical noxious stimulus. Venous blood samples were regularly collected to determine alfaxalone plasma concentrations and for pharmacokinetic analysis. Cardiopulmonary variables were monitored and the quality of the anaesthetic recovery period scored.

Results

The median (range) alfaxalone infusion rate was 3.1 (2.4 – 4.3) mg kg^{-1} $hour^{-1}$. The mean (± SD) plasma elimination half-life, plasma clearance and volume of distribution for alfaxalone were 41 minutes, 25 (± 6.3) mL $minute^{-1}$ kg^{-1} and 1.6 (± 0.5) L kg^{-1}, respectively. During anaesthesia, mean arterial blood pressure was maintained above 70 mm Hg in all horses. Cardiac index reached a

minimum value (68% of baseline values) immediately after induction of anaesthesia and was maintained between 74 and 90% of baseline values for the remainder of the anaesthetic. Following the cessation of the alfaxalone infusion, 6 of 8 horses exhibited muscle tremors and paddling. All horses stood without incident on the first or second attempt with a median recovery score of 4.5 (good to excellent).

Conclusions

Anaesthesia in horses can be maintained with an infusion of alfaxalone at approximately 3 mg kg^{-1} $hour^{-1}$. The alfaxalone infusion rates used resulted in minimal haemodynamic changes and good recovery quality. Mean alfaxalone plasma concentration was stable over the infusion period and clearance rates were similar to a previously published single dose alfaxalone studies in horses.

Introduction

Alfaxalone formulated in 2-hydroxypropyl-beta-cyclodextrin (HPCD), is a neurosteroid molecule that interacts with the gamma aminobutyric acid $(GABA)_A$ receptor to produce anaesthesia and muscle relaxation. In horses, alfaxalone has been shown to have a number of pharmacodynamic and pharmacokinetic properties that suggest it is suitable to for maintenance of total intravenous anaesthesia (TIVA). For example, following administration of a single dose to premedicated adult horses and foals, alfaxalone has been shown to have a rapid onset of action, result in a short duration of recumbency and satisfactory recoveries from anaesthesia. Additionally in horses and foals alfaxalone is rapidly cleared from the plasma with plasma clearances reported to be 37 and 20 mL $minute^{-1}$ kg^{-1}, respectively (Goodwin et al. 2011; Goodwin et al. 2012).

Alfaxalone infusions have been used alone or with various combinations of other infused drugs such as medetomidine, guaifenesin and butorphanol to maintain anaesthesia in horses and ponies undergoing castration for up to 60 minutes (Goodwin et al. 2013; Ohmura et al. 2016; Aoki et al.

2017; Deutsch et al. 2017). These studies concluded that alfaxalone, when used alone or combined with other drugs, was suitable for short term anaesthetic maintenance in horses and ponies; however there is no information regarding the use of alfaxalone as the sole agent to maintain anaesthesia for periods longer than 60 minutes.

The primary objective of this study was to determine if alfaxalone could be used as the sole agent to maintain anaesthesia in horses for 180 minutes and to concurrently report the alfaxalone infusion rates, the cardiovascular effects during the alfaxalone infusion, the quality of the anaesthetic recovery period and the pharmacokinetics.

Materials and Methods

Animals

This study was performed with approval of The University of Queensland Animal Ethics Committee (SVS/435/06/UQ). Eight standardbred horses (4 mares, 4 geldings) with mean (± SD) age and weights of 9 (± 2) years and 441 (± 40) kg, respectively, from The University of Queensland Research Herd were included in the study. Horses were considered healthy on the morning of the study based on physical examination and haematological and blood biochemical analysis.

Instrumentation

A 3.25 inch 14 gauge catheter (BD Angiocath; Becton Dickinson Inc., UT, USA) was placed in the right jugular vein for drug administration and a second catheter placed in the left jugular vein for venous blood collection. A 20 gauge 2 inch catheter (Optiva, Smiths Medical Australasia Pty Ltd, NSW, Australia) was placed in the left carotid artery (previously relocated subcutaneously) for arterial blood pressure monitoring and collection of blood for gas analysis. Two 8.5 French gauge introducers (Percutaneous Sheath Introducer Set, Arrow International, OH, USA) were placed in the

right jugular vein approximately 15 cm apart. A 7 French gauge, 110 cm, thermodilution catheter (Hands-Off Heparin Coated Thermodilution Catheter, Arrow International, OH, USA) was placed via the distal introducer into the pulmonary artery (PA) and an 8 French gauge 70 cm catheter (Devon open-end catheter, Devon Innovations, India) was placed via the proximal introducer into the right atrium (RA) to administer the injectate for the cardiac output (CO) determination and to measure central venous pressure (CVP). Catheters were connected via saline solution filled lines to pressure transducers (Single transducer BD BTXPlus, Becton Dickinson Inc, Singapore) and a Marquette Eagle 4000 Patient Monitor (Marquette Electronics Inc, WI, USA). Correct positioning was confirmed by characteristic blood pressure waves. The left carotid catheter was connected via a saline solution filled line to a transducer (MLT0670 Disposable BP Transducer, ADInstruments, NSW, Australia) and a Powerlab data acquisition system (ADInstruments Pty Ltd, NSW, Australia). Transducers were zeroed at the level of the shoulder for standing horses and the sternum for anaesthetised horses.

Anaesthetic protocol

After instrumentation, horses were premedicated with acepromazine 0.03 mg kg^{-1} IV (A.C.P. 10, Delvet Pty. Ltd., NSW, Australia) followed 20 minutes later by xylazine 1 mg kg^{-1} IV (Xylazil 100, Troy Laboratories Pty. Ltd., NSW, Australia). Five minutes later horses received guaifenesin (35 mg kg^{-1} IV) (Giafen, Parnell Pty. Ltd., NSW, Australia) and alfaxalone (1 mg kg^{-1} IV) (Alfaxan, Jurox Pty. Ltd, NSW, Australia). The time from end of the alfaxalone administration to when horses became laterally recumbent was recorded. The quality of induction was assessed by a veterinarian experienced in equine anaesthesia as previously described (1-5 with 5 representing optimal induction) (Goodwin et al. 2011).

Once recumbent, horses were positioned in left lateral recumbency on a high density foam mattress and the trachea intubated with a 28 mm internal diameter cuffed endotracheal tube. The ease of

intubation was scored on a scale of 1-3, with 3 being optimal. Horses were connected to a large animal circle system (Easy Veterinary Equipment, NSW, Australia) and oxygen supplied at 6 L minute^{-1}. Mechanical ventilation (MV) commenced if horses became apnoeic (no spontaneous ventilation for 60 seconds) or if $PaCO_2$ was above 70 mmHg (9.3 kPa). Mechanical ventilation was performed manually with a 30 L rebreathing bag with an inspiratory time of 2 seconds and a peak inspiratory pressure of 20 cmH_2O. Frequent attempts were made to restore spontaneous ventilation; however these attempts were abandoned if $PaCO_2$ remained above 70 mmHg (9.3 kPa). The urinary bladder was catheterised to permit continuous drainage during anaesthesia.

Anaesthesia was maintained with an infusion of alfaxalone (5 mg mL^{-1} in 0.9% Sodium Chloride; Baxter Healthcare Pty. Ltd., NSW, Australia) for 180 minutes. Based on data from preliminary trials the alfaxalone infusion was started at 5 mg kg^{-1} hour^{-1} delivered by a calibrated infusion pump (IMED Gemini PC-1 infusion pump, Alaris Medical Systems Inc, CA, USA) (Goodwin 2013). The alfaxalone infusion rate was adjusted based on the horses' response to a supramaximal noxious stimulus that was applied approximately every 20 minutes after induction of anaesthesia. Two surface electrodes (inter-electrode distance of 1 cm) were applied to the oral mucous membranes to deliver an electrical stimulation (50V at a frequency of 5 Hz, 10 milliseconds) for 60 seconds. A response was considered positive if purposeful, gross movement of the head or limbs occurred. If no positive response occurred within 60 seconds of applying the electrical stimulus the alfaxalone infusion was decreased by 30% of the preceding infusion rate. Conversely, if a positive response occurred the alfaxalone infusion was increased by 30% of the preceding infusion rate. For each horse, the infusion rates and any bolus doses of alfaxalone administered were recorded and used to calculate the total alfaxalone administered during the infusion period. The time intervals from the end of anaesthesia (cessation of alfaxalone infusion) to tracheal extubation (swallowing), first head lift, sternal recumbency and standing were recorded. The quality of recovery from anaesthesia was

scored as previously described (1-5 with 5 representing optimal recovery) (Goodwin et al. 2011). Once horses were extubated, oxygen was insufflated via an intranasal tube at 15 L minute^{-1}.

Cardiopulmonary Measurements

Cardiopulmonary variables were recorded before drug administration (baseline), immediately prior to induction of anaesthesia (post premedication), within 10 minutes of induction of anaesthesia (induction) and then every 20 minutes just prior to application of electrical stimulation for the remainder of the anaesthetic period. A continuous electrocardiogram (ECG) (base-apex lead), heart rate (HR), direct arterial blood pressure and respiratory rate (f_R) were recorded using the Powerlab data acquisition system. Arterial blood samples were collected anaerobically from the carotid artery and analysed immediately using a Vetstat Analyser (Idexx Laboratories, Westbrook, ME, USA) for pH, PaO_2, $PaCO_2$ and HCO_3. Cardiac output was measured using the thermodilution technique with 30 mL of 0°C 5% dextrose manually injected over approximately 3 seconds through the RA catheter at the end of expiration. The temperature fluctuation was measured using the thermodilution catheter positioned in the PA and CO calculated by the monitor software. Cardiac output and CVP measurements were made three times at each time point and the average recorded. Cardiac Index (CI), systemic vascular resistance (SVR) and stroke volume (SV) were calculated using standard formulae (Scharzwald et al. 2009).

Determination of plasma alfaxalone concentration

Venous blood samples were collected before premedication and at 2, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 minutes after the induction dose of alfaxalone had been administered for the determination of plasma alfaxalone concentrations. Sample collection, storage, processing and plasma extraction were as previously described (Goodwin et al. 2011). Plasma alfaxalone concentrations were determined using a liquid chromatography/tandem mass spectrometry assay validated in horses (Goodwin et al. 2011).

Pharmacokinetic analysis

For plasma alfaxalone concentration, non-compartmental pharmacokinetic analysis using WinNonlin® Professional (version 5.3; Pharsight Corp., St. Louis, MO, USA) was used to calculate secondary pharmacokinetic variables including area under the curve to the last quantifiable time point ($AUC_{0\text{-}Tlast}$), area under the curve to infinity ($AUC_{0\text{-}inf}$), area under the curve percentage extrapolated (AUC_{extrap}), volume of distribution based on terminal elimination phase (V_z), volume of distribution at steady state (V_{ss}), total body clearance (Cl), terminal elimination first order rate constant (lambda_z) and terminal elimination half-life ($t_{1/2\_Lambda\_z}$). Maximum observed plasma alfaxalone concentration (C_{max}) and time at maximum observed plasma alfaxalone concentration values (T_{max}) were taken directly from the individual plasma alfaxalone over time curves while $AUC_{0\text{-}Tlast}$, $AUC_{0\text{-}inf}$ and AUC_{extrap}, V_z, V_{ss}, Cl, lambda_z and $t_{1/2\_Lambda\_z}$ were calculated/estimated (Table 1).

Plasma alfaxalone concentration values < LLOQ (lower limit of quantification) were assumed to be zero when they occurred before the first value > LLOQ. The harmonic mean was calculated using the HARMEAN() function in Microsoft Office Excel 2007 (Microsoft Corporation, WA, USA): Harmonic mean = $N/(1/a_1 + 1/a_2 \ldots + 1/a_N)$ where N is the number of data points and "a" is an individual data point.

Statistical Analysis

Data is presented as median and ranges and pharmacokinetic parameters as mean, standard deviation and range. For cardiopulmonary variables, line graphs were used to analyse changes over time, box plots to assess the presence of outliers and Shapiro-Wilk's test to check for normality. Normally distributed variables (pH and HCO_3-) at baseline and then at designated time points after premedication and induction of anaesthesia were compared using repeated measures ANOVA with

post hoc Tukey test with Bonferroni correction. All other cardiopulmonary variables (not normally distributed) at baseline and then at designated time points after premedication and induction of anaesthesia were compared using the Friedman's test. Level of significance for all tests was $p < 0.05$. All calculations were performed using R version 3.42 (R Foundation for Statistical Computing, Vienna, Austria). A sample size of eight horses was used as this was the maximum number of animals able to be accommodated with the available resources.

Results

Induction of anaesthesia

Following induction of anaesthesia with alfaxalone, the median (range) time taken for horses to become laterally recumbent was 53 (38-70) seconds. Induction of anaesthesia was smooth for all horses and the median score for quality of anaesthetic induction was 5 (excellent) (4 - 5). The ease of endotracheal intubation was scored as 3 (no swallowing, intubated easily) for all horses.

Infusion of alfaxalone

The alfaxalone infusion started 1.75 (1.2 - 6.1) minutes after the end of the alfaxalone induction injection and was infused for 180.5 (178.9 - 182.6) minutes at rate of 3.1 (2.4 – 4.3) mg kg^{-1} $hour^{-1}$. Individual horse alfaxalone infusion rates and response to noxious stimulus are shown in Table 2. Most horses demonstrated brisk palpebral reflexes, intermittent nystagmus and swallowing throughout anaesthesia but this was not consistently associated with gross purposeful movement. Positive reactions mostly consisted of gentle limb and head movement or slow paddling of all four limbs.

Alfaxalone plasma levels and pharmacokinetics

The pharmacokinetic estimates for individual horses are shown in Table 3. The mean (± SD) Cl, Vd and $t_{1/2elim}$ was 25 ± 6.3 mL $minute^{-1}$ kg^{-1}, 1.57 ± 0.53 L kg^{-1} and 41 minutes, respectively. After the

infusion was stopped, alfaxalone was quantifiable up to 120 minutes in four horses, 180 minutes in three horses and 300 minutes in one horse. The individual alfaxalone plasma concentrations over time for individual horses are shown in Figure 1.

Haemodynamic variables

No ECG abnormalities were observed during the anaesthetic period. Overall, there was a significant difference for all cardiovascular variables when the infusion period was compared with baseline values; however, smaller but still significant differences were noted when baseline values were compared with values for each variable taken every 20 minutes during the infusion (Table 4). Although not statistically significant, CI reached a minimum value immediately after induction of anaesthesia (68% of baseline values) and was maintained between 74 and 90% of baseline values for the remainder of the anaesthetic. Stroke volume and CVP trended downwards as the infusion progressed. Systemic vascular resistance tended to increase during the infusion period and was significantly increased from baseline at 100 ($p = 0.045$), 120 ($p = 0.045$) , 160 ($p = 0.045$) and 180 ($p = 0.044$) minutes. Arterial blood pressure and heart rate also trended upwards towards the end of the infusion. Mean arterial pressure (MAP) and DAP were significantly different from baseline at 60 and 80 minutes ($p = 0.04$) and 40, 60, 140, 160 and 180 minutes ($p = 0.038$), respectively.

Respiratory rate and arterial blood gases

Six horses became apnoeic during anesthesia and required MV for the majority of the anaesthetic period. As for cardiovascular variables, there was an overall significant difference for respiratory rate and arterial blood gases when the infusion period was compared with baseline values; however smaller but still significant differences were noted when baseline values were compared with values for each variable taken every 20 minutes during the infusion (Table 5). Significant increases in $PaCO_2$ ($p = 0.04$) and HCO_3- ($p < 0.01$) and decreases in pH ($p < 0.01$) values compared with baseline were observed from 20 – 180 minutes of the infusion.

Recovery

The time to extubation after discontinuation of the infusion was 5 (1-14) minutes. Time to first head lift, achieving sternal recumbency and standing was 18 (12-64), 65.5 (35-118) and 90.5 (64-129) minutes, respectively. Approximately 10 to 20 minutes after the cessation of the alfaxalone infusion, six of eight horses exhibited some form of central nervous system excitation that ranged from mild muscle tremors and twitching to profound paddling and muscle rigidity. These episodes were intermittent over a maximum period of 25 minutes and appeared to be precipitated by noise and tactile stimuli. Despite this, all horses stood without incident on the first or second attempt and the median recovery score was 4.5; good to excellent (range 3 - 5).

Discussion

The results of this study suggest an infusion of alfaxalone can be used as the sole agent to maintain TIVA for 180 minutes in horses. The anaesthetic technique resulted in a smooth anaesthetic induction and good preservation of cardiovascular function. Recovery from anaesthesia was satisfactory; however, some horses exhibited muscle tremors and paddling soon after the alfaxalone infusion was ceased.

During the study, horses were electrically stimulated to mimic a surgical stimulus and the alfaxalone infusion rate adjusted in an attempt to achieve a light depth of anaesthesia. Due to the experimental design, anaesthesia would have been inadequate for clinical use as all horses moved at some stage during the alfaxalone infusion in response to stimulation. Also, some unstimulated horses moved spontaneously (Table 2) and it was often difficult to judge the depth of anaesthesia and anticipate a response to stimulation. For example, despite displaying brisk palpebral reflexes, nystagmus and swallowing, horses would often not move in response to stimulation. Brisk palpebral movements, nystagmus and skeletal muscle twitching not associated with gross purposeful movement or depth of anaesthesia was reported in goats receiving infusions of alfaxalone during

determination of minimum infusion rates (MIR) (Ndawana et al. 2015). The minimum infusion rate is a concept similar to minimum alveolar concentration (MAC) and is defined as the infusion rate that will prevent movement in response to surgical stimulation in 50% of patients (Prys-Roberts 1980). This study was not designed to determine the MIR; however, horses were lightly anaesthetised and it is possible the calculated infusion rates may more closely approximate the MIR than alfaxalone infusion rates that are appropriate for clinical use in horses.

Previously, lower alfaxalone infusion rates (1.5 – 2 mg kg^{-1} $hour^{-1}$) co-infused with various combinations of medetomidine, guaifenesin and butorphanol were reported to provide an adequate depth of anaesthesia for horses anaesthetised for 45 - 60 minutes during castration surgery (Goodwin et al. 2013; Ohmura et al. 2016; Aoki et al. 2017). In retrospect, the initial alfaxalone infusion rate of 5 mg kg^{-1} $hour^{-1}$ used in this study was too high as most horses did not respond to the noxious stimulus until they were receiving considerably lower infusion rates (1.25 – 1.75 mg kg^{-1} $hour^{-1}$) approximately 60 minutes after the infusion began. This was likely due to the effects of the acepromazine, xylazine and guaifenesin acting additively and/or synergistically with alfaxalone to lower the total amount of alfaxalone required to maintain anaesthesia. It is also possible that these drugs altered haemodynamics, drug perfusion and the redistribution of alfaxalone and the concentration of the drug at the target receptor site. Consequently, in future clinical studies it will be important to account for the effects of premedication by starting at lower alfaxalone infusion rates and possibly incorporating a “step-up” process that sequentially increases the infusion rate during the first 60 minutes of anaesthesia.

Maintenance of adequate cardiovascular function is essential during anaesthesia of horses. In this study CI was well maintained during anaesthesia and ranged from 48 to 63 mL $minute^{-1}$ kg^{-1} representing a reduction from baseline values of approximately 32 to 10%. The greatest reduction in CI was noted immediately after induction of anaesthesia (32%) and

for the remainder of the anaesthetic period CI was within approximately 15% of baseline values. Heart rate was also well maintained and tended to increase during the infusion and may have helped to maintain CI. It is likely that the premedicants in this study contributed to the drop in CI after induction of anaesthesia and early into the anaesthetic maintenance period. The administration of the same dose of xylazine to standing horses has been shown to reduce CI by approximately 50% with the effects greatest 5 minutes after administration and continuing to significantly reduce CI from baseline values for 40 minutes (Yamashita et al. 2000).

A MAP above 70 mmHg is considered necessary to prevent the development of postoperative myopathies in anaesthetised horses (Duke et al. 2006) and all horses in the current study maintained MAP above 70 mmHg without the use of sympathomimetic drugs. Systemic vascular resistance was also well maintained and significantly increased compared with baseline values towards the end of the infusion; therefore, suggesting that minimal vasodilation occurred. These findings are in agreement with studies of alfaxalone in other species, such as cats, dogs and sheep, where good haemodynamic stability has also been reported (Ambros et al. 2008; Muir et al. 2008; Muir et al. 2009; Granados et al. 2012).

In the current study, six of eight horses became apnoeic and required MV. Mechanical ventilation was discontinued at intervals as the authors were interested to observe if adequate ventilation would occur when horses were lightly anaesthetised. However, adequate spontaneous ventilation did not occur and consequently for the majority of the anaesthetic period respiratory depression was evident. Dose dependent respiratory depression has been described in dogs and cats receiving a single bolus or an infusion of alfaxalone (Ambros et al. 2008; Muir et al. 2009; Keates & Whittem 2012); however, definitive conclusions regarding the respiratory effects of alfaxalone on horses cannot be drawn from this study. It is noted that interpretation of the haemodynamic variables in this study may have been confounded by the indirect sympathomimetic effects of increased $PaCO_2$

313 which is generally associated with improved haemodynamics (Wagner et al. 1990). Conversely, the
314 use of MV can cause detrimental cardiovacular effects and may have negatively influenced
315 haemodynamic variables in this study (Hodgson et al. 1986).
316
317 While FiO_2 was not recorded, PaO2 was generally well maintained, although lower than expected
318 for horses breathing 100% oxygen via a circle system. This may have been due to hypoventilation,
319 however, other factors commonly associated with recumbency and general anaesthesia in horses,
320 such as ventilation-perfusion mismatching and intra-pulmonary shunts, are also likely to have
321 contributed to the lower PaO_2 values observed (Nyman & Hedenstierna 1989; Nyman et al. 1990).
322 Further work to investigate the pulmonary effects of alfaxalone in horses is required; however,
323 based on these preliminary findings, the authors recommend oxygen supplementation and provision
324 for MV if anaesthesia is maintained with an infusion of alfaxalone alone.
325
326 The pharmacokinetic profile of alfaxalone in this study was similar to that reported after a single
327 induction dose of alfaxalone in adult premedicated horses (Goodwin et al. 2011). Following 180
328 minutes of infusion, alfaxalone was rapidly cleared from horse plasma at approximately 25 mL
329 $minute^{-1}$ kg^{-1}. This corresponds to approximately 32% of CO and represents only a modest
330 reduction to plasma clearance after a single bolus dose (37 mL $minute^{-1}$ kg^{-1}, 46% of CO). The
331 average volume of distribution (1.6 L) was the same as previously reported for a single bolus dose.
332 The plasma elimination half-life of alfaxalone after 180 minutes of infusion was only slightly
333 increased to that reported after a single dose (41 minutes vs 33 minutes). This fact in concert with
334 similar alfaxalone clearance rates in the single dose and infusion horse studies shows that alfaxalone
335 is cleared by first order kinetics even after 180 minutes of infusion.
336
337 Horses recovered from anaesthesia to the standing position smoothly with few attempts and was of
338 a similar quality to anaesthetic recoveries described after short term alfaxalone anaesthesia (Leece

et al. 2009; Goodwin et al. 2011; Kloppel & Leece 2011; Goodwin et al. 2013). In this study, the time taken for horses to achieve the standing position was longer than that reported after a single dose in premedicated horses (90.5 minutes vs 47 minutes) (Goodwin et al. 2011). As the mean elimination half-life of alfaxalone in this study was similar to that observed in the aforementioned single dose study, it is likely that the longer recovery times are due to changes in physiology induced by protracted anaesthesia (e.g. decreased core body temperature, reversible muscle compartmental syndrome). For example, in cats with anaesthesia maintained with a target controlled infusion of alfaxalone, the context sensitive half time was short regardless of the duration of the infusion, however the recovery time was predicted to be influenced by the duration of the infusion (Pypendop et al. 2018).

Although recovery from anaesthesia to the standing position was satisfactory, early in the recovery period most horses demonstrated a form of central nervous system excitation as evidenced by paddling, muscle twitching, and an exaggerated response to noise and handling. Similar observations during the anaesthetic recovery period following alfaxalone infusions have also been reported in cats (Schwarz et al. 2014). It is interesting that central excitation was not observed in horses receiving lower alfaxalone infusion rates (1.5 - 2 mg kg^{-1} $hour^{-1}$) co-infused with medetomidine alone, medetomidine and guaifenesin or medetomidine and butorphanol for 45 - 60 minutes (Goodwin et al. 2013; Ohmura et al. 2016; Aoki et al. 2017). However, in later preliminary trials, we again observed a similar degree of central nervous system excitation during the recovery period of three horses receiving co-infusions of alfaxalone (1.4 - 3.6 mg kg^{-1} $hour^{-1}$) and medetomidine (3.5 - 5 mg kg^{-1} $hour^{-1}$) for 110-180 minutes (Goodwin 2013). We hypothesised that the central nervous effects may be linked to synaptic plasticity and neuromodulation at the $GABA_A$ and glycine receptor. Lau et al (2010) reported that in an *in vitro* hypoglossal nerve model, alfaxalone reduces glycinergic inhibitory transmission which made the nerve more “accessible” to

364 excitatory neurotransmission. Clearly, more research is required to elucidate the temporal effects of
365 alfaxalone and associated endogenous ligands on the $GABA_A$ and glycine receptor.
366
367 Limitations of this study include the small number of horses studied and that the 30% change
368 (increase or decrease) in alfaxalone infusion rate from the preceding rate may have been too small
369 to result in appreciable differences in concentrations of the drug at the target receptor. In hindsight,
370 the study may have been designed to determine the MIR of alfaxalone in horses with the blood
371 sampling for plasma alfaxalone analysis timed so that Cp50 could also be determined. This was not
372 done as the authors were more interested in the clinical application of alfaxalone infusions in horses
373 and to determine alfaxalone infusion rates to be used in a later MAC reduction study (Goodwin
374 2013).
375
376 In conclusion, alfaxalone infused at approximately 3 mg kg^{-1} hr^{-1} for 180 minutes resulted in a
377 relatively stable maintenance phase of anaesthesia with minimal haemodynamic changes.
378 Mechanical ventilation and supplemental oxygen were required to overcome respiratory depression
379 and reduce the incidence of low blood oxygenation. Most horses stood calmly on the first attempt
380 although there was a high incidence of central nervous system excitation in the period immediately
381 following the cessation of the infusion. Mean alfaxalone plasma concentration was stable over the
382 infusion period and clearance rates were similar to a previously published single dose alfaxalone
383 study in horses.
384
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Table 1 Pharmacokinetic definitions and how they were calculated for 8 Standardbred horses premedicated with intravenous (IV) acepromazine (0.03 mg kg^{-1}) and xylazine (1 mg kg^{-1} IV) and anaesthesia induced with guaifenesin (35 mg kg^{-1} IV) and alfaxalone (1 mg kg^{-1} IV) and maintained with a variable rate infusion of alfaxalone for 180 minutes.

Pharmacokinetic variable	**Calculation methods**
Area under the curve from time of drug administration to the last quantifiable time point (**$AUC_{0\text{-}Tlast}$**) (minute mg L^{-1})	Calculated using the linear trapezoidal rule (Gibaldi & Perrier 1982)
Area under the curve from time of drug administration to infinity (**$AUC_{0\text{-}inf}$**) (minute mg L^{-1})	$AUC_{0\text{-}inf} = AUC_{0\text{-}Tlast} + lambda\_z/C_{last}$ Where C_{last} equals the last quantifiable alfaxalone plasma concentration.
Percentage of $AUC_{0\text{-}inf}$ that is due from T_{last} (i.e. time of last quantifiable alfaxalone concentration) to infinity (**AUC_{extrap}**) (%)	$AUC_{extrap}(\%) = AUC_{0\text{-}Tlast} \div AUC_{0\text{-}inf} \times 100\%$.
Volume of distribution based on terminal phase lambda_z (**Vz**) (L kg^{-1})	$$Vz = \frac{Dose}{\lambda z \times AUC_{0-inf}}$$
Volume of distribution at steady state (**Vss**) (L kg^{-1})	$$Vss = \frac{Dose \times AUMC}{(AUCzero - \tau)^2}$$ Where AUMC is the area under the first moment curve and τ (Tau) is the dosing interval.
Total body clearance (**Cl**) (mL $minute^{-1}$ kg^{-1})	$$Cl = \frac{Dose}{AUC_{0-inf}}$$
First order rate constant associated with the terminal portion of the curve (**Lamba_z**) (mL $minute^{-1}$)	The estimate lambda_z was calculated by linear regression on the elimination portion of the curve using a best fit approach where n is the number of data points in the regression and R^2 is the square of the correlation coefficient. For each regression an adjusted R^2 was computed with: $$Adjusted\ R^2 = \frac{1 - (1 - R^2)\ x\ (n - 1)}{n - 2}$$
Terminal half-life (**$t_{1/2\_Lambda\_z}$**) (minutes)	$t_{1/2\_Lambda\_z} = \ln(2)/\lambda z$

Table 2 Alfaxalone infusion rates (mg kg^{-1} hr^{-1}), response to nociceptive testing and average infusion rate for 8 Standardbred horses premedicated with intravenous (IV) acepromazine (0.03 mg kg^{-1}) and xylazine (1 mg kg^{-1} IV) and anaesthesia induced with guaifenesin (35 mg kg^{-1} IV) and alfaxalone (1 mg kg^{-1} IV) and maintained with a variable rate infusion of alfaxalone for 180 minutes.

Horse 1		**Horse 2**		**Horse 3**		**Horse 4**		**Horse 5**		**Horse 6**		**Horse 7**		**Horse 8**	
Time (minutes)	Infusion rate	Time (minutes)	Infusion rate	Time (minutes)	Infusion rate	Time (minutes)	Infusion rate	Time (minutes)	Infusion rate	Time (minutes)	Infusion rate	Time (minutes)	Infusion rate	Time (minutes)	Infusion rate
1.4	5	1.2	5	2	5	1.5	5	2.4	5	2.4	5	6.1	5	1.4	5
24$^{\vee}$	3.5	24$^{\vee}$	3.5	23$^{\vee}$	3.5	23$^{\vee}$	3.5	26$^{\vee}$	3.5	24$^{\vee}$	3.5	26$^{\vee}$	3.5	24$^{\vee}$	3.5
44$^{\vee}$	2.5	43$^{\vee}$	2.5	44$^{\vee}$	2.5	43$^{\vee}$	2.5	45$^{\vee}$	2.5	44$^{\vee}$	2.5	45$^{\vee}$	2.5	43$^{\vee}$	2.5
61$^{\vee}$	1.75	63$^{\vee}$	1.75	64$^{\vee}$	1.75	64$^{\vee}$	1.75	65$^{\vee}$	1.75	64$^{\vee}$	1.75	66$^{\vee}$	1.75	64$^{\vee}$	1.75
83$^{\wedge}$	2.5	83$^{\vee}$	1.25	82$^{\wedge}$	2.5	85$^{\vee}$	1.25	86$^{\vee}$	1.25	83$^{\wedge}$	2.5	86$^{\vee}$	1.25	*86$^{\wedge}$	2.5
103$^{\vee}$	1.75	104$^{\wedge}$	1.75	104$^{\wedge}$	3.5	106$^{\wedge}$	1.75	89$^{\#\wedge}$	1.75	92$^{\wedge}$	3.5	105$^{\wedge}$	1.75	105$^{\wedge}$	3.5
123$^{\wedge}$	2.5	115$^{\#\wedge}$	2.5	124$^{\vee}$	2.5	125$^{\vee}$	1.25	96$^{\#\wedge}$	2.5	104$^{\wedge}$	5	112$^{\#\wedge}$	2.5	125$^{\wedge}$	3.5
143$^{\vee}$	1.75	124$^{\wedge}$	3.5	145$^{\wedge}$	3.5	143$^{\wedge}$	1.75	115$^{\#\wedge}$	3.5	125$^{\wedge}$	6.5	127$^{\wedge}$	3.5	146$^{\vee}$	2.5
163$^{\wedge}$	2.5	135$^{\vee}$	5	164$^{\vee}$	2.5	163$^{\wedge}$	2.5	126$^{\vee}$	2.5	144	6.5	144$^{\wedge}$	5	160$^{\wedge}$	3.5
183$^{\vee}$	2.5	144$^{\vee}$	3.5	182$^{\wedge}$	2.5	182$^{\vee}$	2.5	147$^{\vee}$	1.75	164$^{\vee}$	5	165$^{\vee}$	3.5	184$^{\vee}$	3.5
		163$^{\wedge}$	5					154$^{\wedge}$	2.5	183$^{\wedge}$	5	185$^{\#\wedge}$	3.5		
		181$^{\vee}$	5					184$^{\vee}$	2.5						
Average alfaxalone infusion mg kg^{-1} hr^{-1}	**2.7**		**3.2**		**3**		**2.4**		**2.8**		**4.3**		**3.1**		**3.5**

Time (min): time from end of alfaxalone induction injection. Infusion rate: alfaxalone infusion rate mg kg^{-1} $minute^{-1}$. ^ Noxious stimulation with positive response. ˇ Noxious stimulation with negative/no response. #^ No stimulation but horse spontaneously moving. *Horse received 100 mg IV alfaxalone as attempting to stand. Average infusion rate for each horse calculated at total alfaxalone dose during infusion (mg)/horse weight (kg)/infusion time (minutes).

Table 3 Pharmacokinetic variables estimated by non-compartmental analysis of alfaxalone concentrations in 8 Standardbred horses premedicated with intravenous (IV) acepromazine (0.03 mg kg^{-1}) and xylazine (1 mg kg^{-1} IV) and anaesthesia induced with guaifenesin (35 mg kg^{-1} IV) and alfaxalone (1 mg kg^{-1} IV) and maintained with a variable rate infusion of alfaxalone for 180 minutes.

Horse ID	**T_{max} (minute)**	**C_{max} (mg L^{-1})**	**$AUC_{0\text{-}Tlast}$ (minute mg L^{-1})**	**$AUC_{0\text{-}inf}$ (minute mg L^{-1})**	**AUC_{extrap} (%)**	**Vz (L kg^{-1})**	**Vss (L kg^{-1})**	**Cl (mL $minute^{-1}$ kg^{-1})**	**lambda_z (mL $minute^{-1}$)**	**lambda_z Half-Life (minute)**
1	180	2.29	507.6	516.6	1.7	1.13	0.89	17.6	15.6	44.5
2	180	2.92	491.9	505.9	2.8	2.32	1.37	21	0.9	76.9
3	30	2.18	407.9	417.6	2.3	1.29	0.78	24.4	18.9	36.6
4	60	2.64	437.5	417.6	0.7	0.77	0.81	18.6	24.3	28.6
5	2	3.01	373	378.1	1.3	1.54	0.93	25.4	16.5	42.1
6	150	2.01	361.2	377.8	4.4	2.24	1.88	37.1	16.6	41.8
7	150	2.30	366.6	377.8	4.8	1.66	1.42	27	16.3	42.5
8	180	1.94	361.8	374.2	3.3	1.62	1.17	29.1	18	38.4
Mean (SD)	116.5 ± 73.8	2.41 ± 0.40	413.4 ± 59.6	420.7 ± 58.7	2.7 ± 1.4	1.57 ± 0.53	1.16 ± 0.38	25 ± 6.3	16.9 ± 4.2	41*
Range	(2-180)	(1.94-3.01)	(374.2-516.6)	(374.2-516.6)	(0.7-4.8)	(0.77-2.32)	(0.78-1.88)	(17.6-37.1)	(0.9-24.3)	(28.6-76.9)

*calculated as harmonic mean. Results expressed as mean ± SD (range). Time of maximum observed plasma concentration (Tmax), observed maximum plasma concentration (Cmax), Area under the curve from time of drug administration to the last quantifiable time point ($AUC_{0\text{-}Tlast}$), Area under the curve from time of drug administration to infinity ($AUC_{0\text{-}inf}$), percentage of $AUC_{0\text{-}inf}$ that is due from T_{last} (i.e. time of last quantifiable alfaxalone concentration) to infinity (AUC_{extrap}), volume of distribution based on terminal phase lambda_z (Vz), volume of distribution at steady state (Vss), total body clearance (Cl), first order rate constant associated with the terminal portion of the curve (Lamba_z) and Terminal half-life ($t_{1/2\_Lambda\_z}$).

Table 4 Cardiac index (CI), systemic vascular resistance (SVR), stroke volume (SV), central venous pressure (CVP), heart rate (HR), mean arterial pressure (MAP), diastolic arterial pressure (DAP) and systolic arterial pressure (SAP) in 8 horses during 180 minutes of alfaxalone total intravenous anaesthesia.

				Minutes after induction of anaesthesia								**Overall summary during infusion**	
	Base-line	**Post premed**	**Induction**	**20**	**40**	**60**	**80**	**100**	**120**	**140**	**160**	**180**	**Median (IQR)**	***p*-value**
CI (mL minute^{-1} kg^{-1}) (n = 8)	65 (59-110)	54 (44-69)	48 (38-60)	60 (49-74)	61 (55-79)	56 (50-76)	58 (49-76)	53 (44-62)	53 (38-68)	60 (46-81)	53 (43-60)	57 (47-68)	56 (39)	< 0.001
SVR (dynes secs cm^{-5}) (n = 7)	243 (111-331)	317 (238-436)	328 (255-465)	256 (197-313)	250 (176-257)	237 (216-294)	270 (248-351)	374* (307-579)	363* (301-644)	362 (209-511)	466* (258-578)	400* (304-564)	311 (395)	< 0.001
SV (mL) (n = 7)	815 (610-1358)	662 (539-700)	626 (379-1009)	684 (580-992)	696 (578-975)	626 (427-778)	539 (447-783)	514 (480-671)	549 (352-644)	570 (434-900)	498 (411-665)	578 (346-655)	611 (610)	< 0.001
CVP (mm Hg) (n = 8)	8 (6-16)	7 (2-13)	13 (7-16)	8 (-3-16)	5 (1-15)	2 (1-15)	2 (-1-13)	2 (0-15)	4 (-2-16)	3 (-3-18)	4 (-5-16)	3 (-2-16)	5 (18)	< 0.001
HR (beats minute^{-1}) (n = 7)	36 (32-44)	33 (28-36)	35 (32-42)	38 (32-40)	38 (30-47)	40 (35-52)	41 (40-59)	40 (37-52)	40 (36-72)	40 (36-76)	45 (38-61)	42 (40-57)	40 (29)	< 0.001

MAP (mm Hg) (n = 7)	101 (80-115)	96 (82-126)	100 (83-120)	91 (73-110)	82 (75-91)	81* (78-87)	98 (76-125)	112 (89-141)	128 (99-141)	128 (96-140)	130 (97-147)	134* (120-153)	101 (65)	< 0.001
DAP (mm Hg) (n = 7)	89 (74-104)	92 (81-111)	95 (80-125)	88 (68-100)	75* (66-84)	77* (71-80)	90 (75-115)	101 (83-120)	114 (93-130)	120* (92-132)	124* (92-134)	124* (110-148)	95 (64)	< 0.001
SAP (mm Hg) (n = 7)	122 (87-133)	109 (82-129)	105 (83-134)	100 (77-135)	92 (81-112)	94 (83-106)	108 (92-157)	127 (100-165)	141 (111-168)	150 (102-168)	135 (100-165)	154 (130-176)	115 (86)	< 0.001

Results at each time point are expressed as median (range). * statistically different compared with baseline values ($p < 0.05$). Summary of overall results as median and interquartile range (IQR) and overall *p*-value reported for anaesthetic period compared with baseline. Baseline values were collected before administration of any drugs, premedication values after xylazine administration and induction values immediately after induction of anaesthesia. Data missing for 1 horse on several variables due to data acquisition failure.

Table 5 Respiratory rate (f_R), arterial pH (pH), arterial pressure of oxygen (PaO_2), arterial pressure of carbon dioxide ($PaCO_2$), and bicarbonate (HCO_3^-) in 8 horses during 180 minutes of alfaxalone total intravenous anaesthesia.

				Minutes after induction of anaesthesia									**Overall summary during infusion**	
	Base-line	**Post premed**	**Induct-ion**	**20**	**40**	**60**	**80**	**100**	**120**	**140**	**160**	**180**	**Median (IQR)**	***p* value**
f_R (breath minute^{-1})	12 (8-16)	14 (10-25)	3 (1-15)	3 (1-4)	4 (2-6)	4 (2-6)	4 (2-13)	4 (2-5)	4 (2-9)	4 (2-16)	4 (2-5)	4 (2-6)	4 (15)	< 0.001
pH	7.45 (7.40-7.47)	7.44 (7.39-7.50)	7.41 (7.39-7.48)	7.29* (7.22-7.34)	7.33* (7.27-7.39)	7.34* (7.27-7.40)	7.35* (7.25-7.42)	7.35* (7.30-7.39)	7.35* (7.30-7.42)	7.31* (7.19-7.38)	7.35* (7.23-7.44)	7.33* (7.22-7.36)	7.36 (0.007)	< 0.001
PaO_2 (mm Hg)	100 (95-113)	91 (75-100)	106 (65-148)	133 (91-276)	242 (108-390)	273 (110-355)	223 (92-389)	309 (127-405)	253 (120-412)	158 (112-385)	190 (93-398)	213 (111-353)	152 (306)	< 0.001
(kPa)	13.3 (12.6-15)	12.1 (10-13.3)	14.1 (8.6-19.7)	17.7 (12.1-36.8)	32.2 (14.4-52)	36.4 (14.6-47.3	31 (12.2-51.8)	41.2 (16.9-54)	33.7 (16-54.9)	21 (14.9-51.3)	25.3 (12.4-53)	28.4 (14.8-47)	20.2 (40.8)	
$PaCO_2$ (mm Hg)	46 (44-48)	47 (42-55)	52 (41-56)	70* (65-86)	62* (59-77)	66* (55-82)	67* (52-75)	65* (55-70)	67* (48-77)	70* (62-101)	66* (51-93)	71* (65-83)	64 (42)	< 0.001
(kPa)	6.1 (5.8-6.4)	6.2 (5.6-7.3)	6.9 (5.4-7.4)	9.3* (8.6-11.4)	8.2* (7.8-10.2)	8.8* (7.3-10.9)	8.9* (6.9-10)	8.6* (7.3-9.3)	8.9* (6.4-10.2)	9.3* (8.2-13.4)	8.8* (6.8-12.5)	9.4* (8.6-11)	8.5 (5.6)	
HCO_3^- (mmolL^{-1})	28.8 (26.6-30.4)	30.1 (27-31.5)	30 (25.9-31)	32* (27.3-32.9)	32* (27.8-32.7)	33* (27.9-35)	33* (30.1-34.9)	32.7* (29.7-36)	32.9* (27.6-35.1)	33.8* (31.3-35.8)	32.6* (30.5-35.9)	34.2* (30.5-35.3)	31.8 (2.4)	< 0.001

Results at each time point are expressed as median (range). * statistically different compared with baseline values ($p < 0.05$). Summary of overall results as median and interquartile range (IQR) and overall *p*-value reported for anaesthetic period compared with baseline. Baseline values were collected before administration of any drugs, premedication values after xylazine administration and induction values immediately after induction of anaesthesia

Figure 1 Alfaxalone plasma levels (mg L^{-1}) over time (minutes) in 8 horses premedicated with intravenous (IV) acepromazine (0.03 mg kg^{-1}) and xylazine (1 mg kg^{-1} IV) and anaesthesia induced with guaifenesin (35 mg kg^{-1} IV) and alfaxalone (1 mg kg^{-1} IV) and maintained with a variable rate infusion of alfaxalone for 180 minutes.